Endosymbiotic bacteria in siphonous green algae exploration of a partnership

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Endosymbiosis is an important driving force behind eukaryotic evolution and led to important key innovations such as the acquisition of mitochondria and chloroplasts. Many algae still host endosymbiotic bacteria which play an important role in various metabolic functions. In the siphonous green alga *Bryopsis* bacteria are present in every phase of the life cycle, indicating an ancient association among host and symbiont, rather than a recent opportunistic and non-specific relationship. To identify the bacterial partner, epiphytes were mechanically removed from *Bryopsis* spp. by a combination of vortexing and the use of glass beads. Cultured epiphytes were sequenced after an initial screening by Rep-PCR and identified as *Alteromonas, Halomonas, Marinobacter, Pseudoalteromonas and Sulfitobacter* spp. Subsequently, different *Bryopsis* samples – cleaned as well as possible – were submitted to a range of molecular techniques such as 16S PCR, cloning, DGGE and RFLP. The clones with bacterial sequences fell apart in different clusters, a representative number of which were sequenced and identified as being *Acinetobacter, Flavobacterium, Glaciecola, Mycoplasma, Planctomyces, Pseudonocardia* en *Roseobacter* spp. In order to examine whether the identified bacteria are in fact endosymbionts, we tried to visualize them by the use of FISH with the universal bacterial probe EUB338. The probe assay was optimized on pure bacterial cultures, both Gram-positives and Gram-negatives. We opted to perform FISH on *Bryopsis* gametes which do not have a cell wall and also contain the endosymbionts. Gametogenesis was effectively generated *in vitro* and the gametes went through different fixation steps, prior to the actual hybridization. Currently the fixation is being optimized.
